Locus-specific chromatin composition analysis by dCas9-driven proximity labelling

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Introduction

Proximity labelling by biotinylation is a powerful tool to study the interaction of proteins in living cells. In this study, we aim to establish a system that allows to directly analyze a genomic locus of choice for the locally positioned proteome and thereby define the chromatin environment of this locus. Special interest is cast on biosynthetic gene clusters (BGCs) that are characterized by a strong dependence on the recently identified KERS complex (Karahoda et al., 2022), facultative heterochromatic marks and by the lack of H3K4 methylation marks despite active gene transcription (Gacek-Matthews et al., 2016).

Here we describe the application of the TurboID-dCas9 fusion protein that can be directed to any desired genomic locus with the goal of biotin labeling chromatin-associated proteins in close proximity to the locus of interest. As a proof of concept, we targeted the well-described *niiA-niaD* bidirectional promoter region in *Aspergillus nidulans* that controls nitrate assimilation genes with several known transcription factors such as AreA and NirA (Berger et al., 2008). Chromatin immunoprecipitation (ChIP) revealed that both TurboID-dCas9 and AreA were present at the AreA binding locus under nitrate-inducing, but not under repressing conditions. In order to detect whether the fusion protein was positioned correctly and working as expected, biotinylation of AreA was verified using a western blot with both anti-HA-antibody and streptactin-HRP. To confirm the correct working mode of the system and to identify other DNA-associated proteins within the proximity of the TurboID-dCas9 annealing site within the *niiA-niaD* promoter, tryptic digest of the enriched biotinylated proteins, followed by LC-MS analysis was carried out. This work presents the concept and first results of locus-specific chromatin composition analysis by proximity biotinylation in *A. nidulans*.

Methods

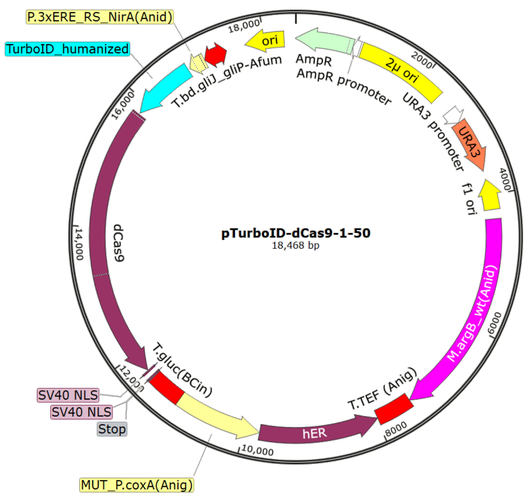

Figure 1: dCas9-TurboID plasmid

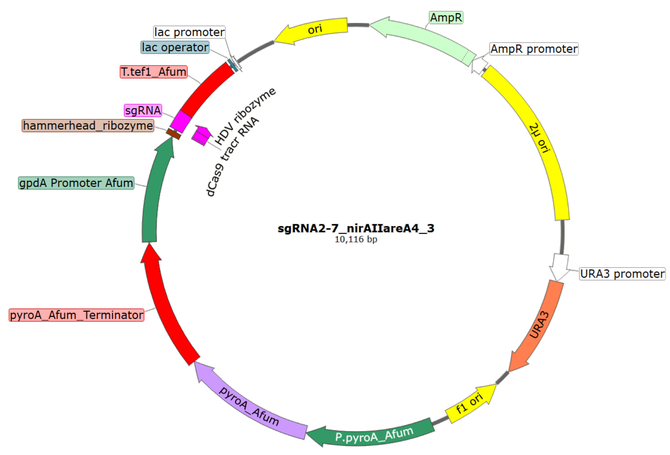

Figure 2: sgRNA plasmid

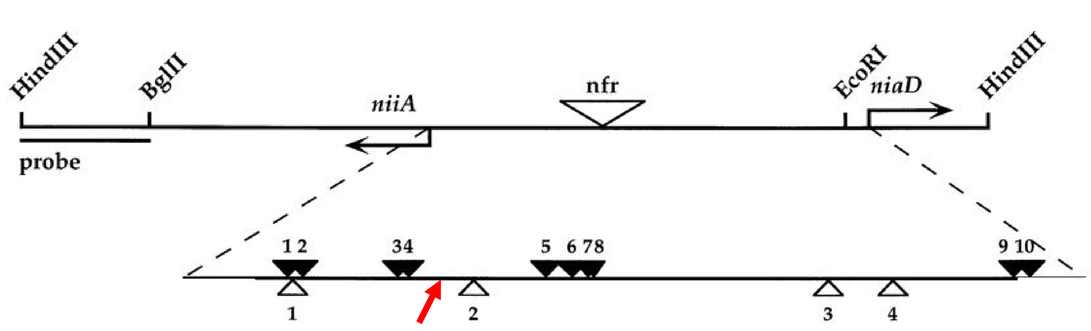

Figure 3: Schematic structure of the *niiA–niaD* bidirectional promoter (modified after Muro-Pastor et al., 1999).
→ White triangles: NirA binding sites
→ Black triangles: AreA binding sites

Strains:

- AreA-HA (background biotinylation)
- dCas9-TurboID without sgRNA (unspecific bitoinylation)
- dCas9-TurboID + sgRNA (specific biotinylation at locus of interest)

Workflow:

- Overnight culture with proline as N-source
- Diethylstilbestrol (DES) added to induce dimerization of human estrogen receptor (hER) → binding to the estrogen responsive element (ERE) to induce transcription of dCas9-TurboID (Figure 4)
- sgRNA guides dCas9-TurboID to a locus between NirA binding site 2 and AreA binding site 4 (Figure 3, red arrow)
- 150 µM $NaNO_3$ supplemented
- 50 µM biotin boost
- Protein extraction
- Enrichment of biotinylated proteins
- LC-MS analysis

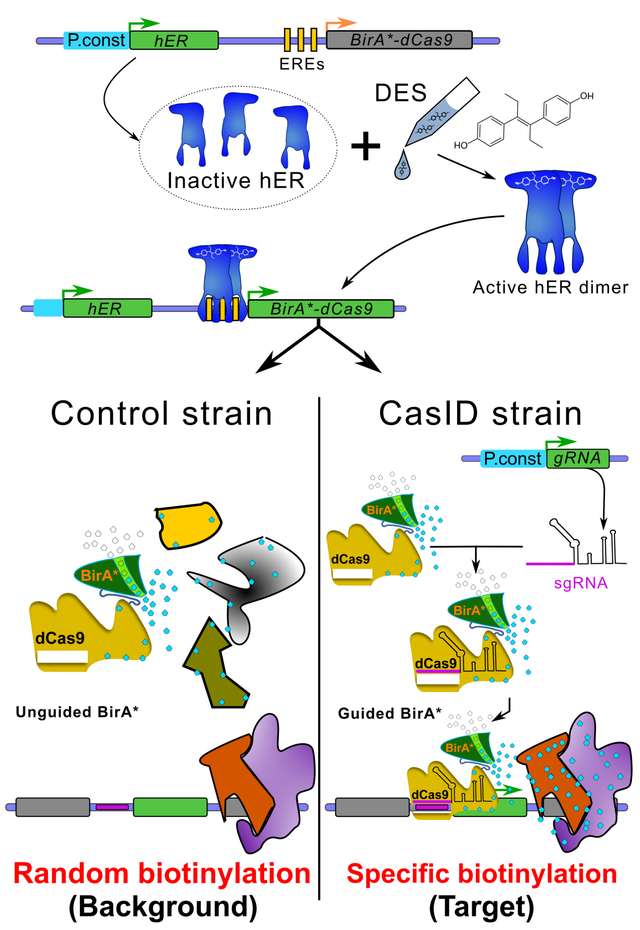

Figure 4: Scheme of biotinylation assay; Induction with DES leads to dimerization of hER enabling binding to ERE inducing transcription. For the test, one strain with, one strain without sgRNA as well as one AreA-HA tagged wild type strain is used.

Results

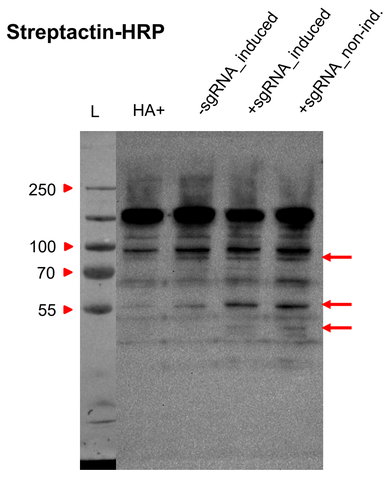

Figure 5: Streptactin western

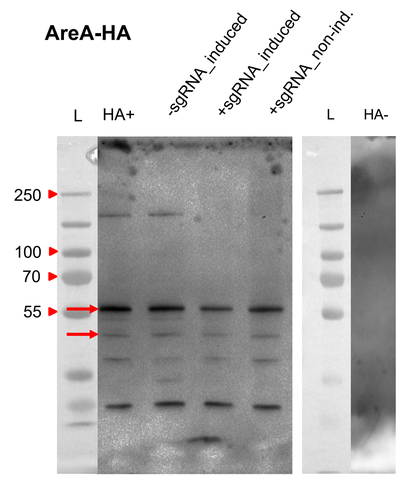

Figure 6: AreA-HA western

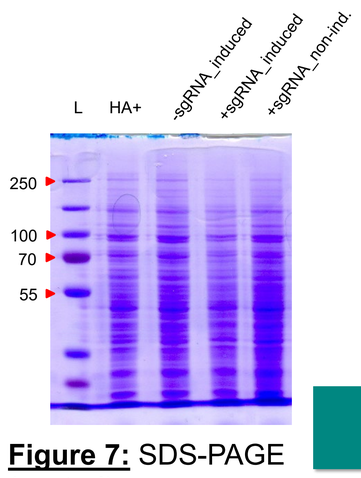

Figure 7: SDS-PAGE (control)

- On the streptavidin-HRP western (Figure 5) there are two bands at 55 kDa and a little below 55 kDa visible (red arrow) corresponding to the AreA bands on the HA western (Figure 6)
- The stronger band at 55 kDa in the strains with sgRNA (Figure 5) indicates that this biotinylation occurred specifically
- A third band (Figure 5) between 70 and 100 kDa is much stronger in the TurboID strains regardless whether they contain the sgRNA
  → indicates unspecific biotinylation during transport to the nucleus
- Biotinylated proteins are enriched via streptavidin speedbeads
- LC-MS analysis in progress

References:

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